DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 4/13/2010 has been entered.

Claims 1, 5, 7-9, 12, 28, 30-33, 37-41, 43, and 45-48 are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 5, 7-9, 12, 28, 30-33, 37-41, 43, and 45-48 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant has amended claims 1 and 9 to recite “wherein the siRNA is capable of single nucleotide discrimination such that expression of the mutant target allele by at least 50% and expression of the wild type allele is not inhibited”. This language renders

the metes and bounds of the claims unclear for the following reason: it is not clear that this language imparts a limitation on the method or describes only a capacity of the siRNA being utilized in the method. For example on could utilize a siRNA capable of promoting this level of inhibition and selectivity, but be used under different conditions where this level of inhibition or selectivity is not observed.

Applicant arguments have been considered. The arguments are not convincing for the reasons set forth below and in view of the new rejection below which address applicants amendments to the claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 5, 7-9, 12, 28, 30-33, 37-41, 43, and 45-48 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Tuschl et al [US 20042059247 A1], Elbashir [The EMBO Journal Vol. 20(23), 2001, cited by applicant as C15 in IDS filed 9/17/07], Brummelkamp et al [Science Vol.296:550-553, April 2002, cited on applicant IDS filed 9/17/2007], Klug et al [European Journal of Physiology, Vol. 441 (6 Suppl): R205, 2001], Brown et al [WO 94/19493], Siddique et al [Neurology Vol. 47(suppl 2): S27-S35, 1996], and Kunst et al [Nature Genetics Vol. 15: 91-94, 01/15/96].

The instant invention is drawn to the inhibition of a SOD1 gain of function allele in a cell via siRNA. The invention includes inhibiting the dominant gain of function allele at least 50% where the wt allele is not inhibited.

Tuschl et al have taught the use of siRNA compound for mediating target-specific RNA interference where the siRNA agents have improved efficacy and safety compared to prior art agents. Tuschl et al have taught: [0030] The target gene to which the RNA molecule of the invention is directed may be associated with a pathological condition. For example, the gene may be a pathogen-associated gene, e.g. a viral gene, a tumor-associated gene or an autoimmune disease-associated gene. The target gene may also be a heterologous gene expressed in a recombinant cell or a genetically altered organism. By determinating or modulating, particularly, inhibiting the function of such a gene valuable information and therapeutic benefits in the agricultural field or in the medicine or veterinary medicine field may be obtained.; [0175] In order to examine the sequence-specificity of target recognition, we introduced sequence changes into the paired segments of siRNA duplexes and determined the efficiency of silencing.

Sequence changes were introduced by inverting short segments of 3- or 4-nt length or as point mutations (FIG. 18). The sequence changes in one siRNA strand were compensated in the complementary siRNA strand to avoid perturbing the base-paired siRNA duplex structure. The sequence of all 2-nt 3' overhangs was TT (T, 2'-deoxythymidine) to reduce costs of synthesis. The TT/TT reference siRNA duplex was comparable in RNAi to the wild-type siRNA duplex AA/UG (FIG. 17). The ability to mediate reporter mRNA destruction was quantified using the translation-based luminescence assay. Duplexes of siRNAs with inverted sequence segments showed dramatically reduced ability for targeting the firefly luciferase reporter (FIG. 18). The sequence changes located between the 3' end and the middle of the antisense siRNA completely abolished target RNA recognition, but mutations near the 5' end of the antisense siRNA exhibit a small degree of silencing. Transversion of the A/U base pair located directly opposite of the predicted target RNA cleavage site, or one nucleotide further away from the predicted site, prevented target RNA cleavage, therefore indicating that single mutation within the centre of a siRNA duplex discriminate between mismatched targets.; [0180] Target recognition is a highly sequence-specific process, mediated by the siRNA complementary to the target. The 3'-most nucleotide of the guide siRNA does not contribute to specificity of target recognition, while the penultimate nucleotide of the 3' overhang affects target RNA cleavage, and a mismatch reduces RNAi 2- to 4-fold. The 5' end of a guide siRNA also appears more permissive for mismatched target RNA recognition when compared to the 3' end. Nucleotides in the centre of the siRNA, located opposite the target RNA cleavage site, are important

specificity determinants and even single nucleotide changes reduce RNAi to undetectable level. This suggests that siRNA duplexes may be able to discriminate mutant or polymorphic alleles in gene targeting experiments, which may become an important feature for future therapeutic developments.

Similarly, Elbashir et al have taught position effects of mismatches in siRNA function. At page 6878 it is taught, for example that target recognition is extremely specific, as even single nucleotide mismatches between the siRNA duplex and the target mRNA abolish interference. And assert that this provide a rational basis for the design of siRNAs. At page 6885 it has been taught that "[n]ucleotides in the center of the siRNA, located opposite to the target RNA cleavage site, are important specificity determinants and even single nucleotide changes reduce RNAi to undetectable levels. This suggests that siRNA duplexes may be able to discriminate mutant or polymorphic alleles in gene targeting experiments, which may become an important feature for future therapeutic developments."

Brummelkamp et al have taught the use of pSuper vectors that utilize pol III promoter to express siRNA as shRNA for the benefit of stable expression of siRNA to mediate persistent suppression of a target gene allowing for the analysis of loss of function phenotypes that developed over an extended period of time. One in the art would clearly have recognized this extended expression benefit in the treatment of disease since one in the art would clearly recognize that the successful treatment of a genetic disease would benefit from administration of a drug that is stably expressed over that of transient administration, for example. The prior art has taught the use of Pol

III promoters such as the recited U6 promoter has been widely used, as the HI exemplified in Brummelkamp. Those in the art are well aware of the benefits of using Pol III promoters to express short RNAs. Brummelkamp et al also assert that their "finding that a single nucleotide mismatch in the 19-nt targeting sequence abrogates the ability to suppress gene expression also opens new avenues for gene therapy. Vectors that target disease-derived transcripts with point mutations, such as those from RAS or TP53 oncogenes, can now be specifically designed, without altering the expression of the remaining wild-type allele. In addition it should be possible to generate large collections of pSUPER siRNA vectors to carry out high-throughput genetic screens for loss of function phenotypes."

Tuschl et al and Elbashir et al and Brummelkamp et al have therefore taught that siRNA can discriminate and inhibit targets with as little as one nucleotide change and have also taught where in the siRNA molecule such changes can be made with the most effective selection of target.

Tuschl et al and Elbashir et al and Brummelkamp et al do not specifically teach targeting gain of function alleles but certainly teach targeting mutant and polymorphic alleles which does embrace dominant gain of function mutations.

Brown et al have taught the involvement of SOD1 and in particular specific mutations in SOD1 that lead to dominant gain of function ALS (see page 28, for example). At pages 10, 31, 53, and claims 43-45 and 47, for example) it is taught to inhibit mutant SOD1 via antisense. In Tables 3A and 3C, the specific mutations targeted

by the instant invention are disclosed [G256C and G281C which correspond to G85Arg and G93A].

Klug et al have taught the targeting of the most common SOD1 mutant gain of function allele G93A with antisense. It was shown the selective inhibition of the mutant allele and uptake of antisense in the brain.

Siddique et al have disclosed SOD1 mutations associated with ALS (see Table, for example).

Kunst et al have also shown that the specific mutant alleles for SOD1 associated ALS were well known at the time of invention (see Figure 1, for example).

The prior art has therefore shown that SOD1 dominant gain of function mutants are causative for ALS. The prior art has shown the targeted inhibition of specific SOD1 alleles via antisense.

The prior art has taught that siRNA can be used to specifically target a desired allele of a gene and that siRNA compounds are more effective inhibitors than antisense [prior art compound of the same class, for example]. The prior art has also taught to specifically inhibit dominant gain of function alleles, including the specific allele of SOD1 recited in the instant claims.

The prior art has shown that mutations associated with ALS (SOD1) have been known for some time before the instant invention. The prior art has also taught to target the mutant alleles of SOD1 selectively over the wt. Since the prior art has also asserted the usefulness of siRNA for treating disease it would have been obvious to use them since the prior art has shown that such targeting was successful using antisense. Since

the sequence of SOD1 and more importantly since the specific mutant sequences were known and the art clearly suggest targeting them, the siRNA sequences of the instant invention are merely optimizations at best.

The prior art taken as a whole shows the instant invention to be obvious at the time of invention even at the levels instantly claimed. For example the prior art has taught to target alleles of SOD1 selectively utilizing antisense oligonucleotides. The art has taught that siRNA compounds can provide a higher level of discrimination than that of antisense compounds. Since the prior art has taught to target even the recited alleles with antisense compounds it is not inventive to utilize a new compound that the prior art indicates is a better compound for such methods. The prior art, evidenced by applicant citations in their response filed 4/09/2010, does show variability in the level of inhibition and discrimination when utilizing mismatched siRNAs. This is not unlike what is shown in applicant own specification. It was well known in the art that there are variations in the effectiveness of any particular siRNA; however the art has shown that one in the art is capable of screening siRNA compounds to find a siRNA that is optimized for its particular application. Since one in the art has been taught that siRNA can discriminate between alleles and since one in the art was apprised of variability, it would not be unexpected to see differences in siRNA effectiveness for any particular target. One in the art would be capable of screening for siRNA compounds that are optimized. The fact that even the particular alleles of SOD1 recited in the claims are taught to be targets for allele discrimination it would not be inventive to screen for an optimized siRNA targeting that site.

The invention as a whole would therefore have been *prima facie* obvious at the time the invention was made.

Response to Arguments

Applicant's arguments filed 4/09/2010 have been fully considered but they are not persuasive Applicant asserts that the claim amendments limit the invention to targeting only single point mutations. This is not what the claims appear to be limited to. The amendments presented in claims 1 and 9 offer that the siRNA "comprises a nucleotide mismatch" and is “capable” of single nucleotide discrimination". The term "comprises" allows for more than one mismatch. This interpretation is evidenced by claim 7, for example which specifically recites “one, two, or three nucleotides”. The term “capable” does not limit the structure of the siRNA, but offers a latent capacity of the compound.

Applicant asserts that the Tuschl and Elbashir references offer no reasonable expectation of success in single nucleotide discrimination between a wild type and mutant allele because they only demonstrate the sequence change effect in silencing a single target sequence. The examiner does not agree with this position, but has provided an additional references to the rejection of record to address this argument

(Brummelkamp et al).Applicant has cited several prior art references which have demonstrated that there is indeed target discrimination in using siRNA with mismatches. Holen is relied on by applicant to assert that only a partial reduction in target disruption was seen with a mutant siRNA comprising one mismatch. The examiner agrees that Holen did not show a complete inhibition of siRNA activity with one mismatch, but there was a decrease in inhibition and therefore there was an observation of target discrimination. The examiner also points out that the observations of Holen et al was based on one siRNA with one mismatch. It is interesting to note that Holen et al assert that target discrimination would be a valuable tool for allele specific inhibition in various dominant negative disorders in cases where low or no tolerance for mismatches was observed.

Jaque et al, Yu et al and Hamada et al are all also relied on by applicant to show that there was variability in the tolerance of mismatches in target discrimination. It is noted by the examiner that all of the reference do, however, show that there was target discrimination in siRNA compounds with as little as one base mismatch. The examiner would note that applicant relies on the TAR and MTAR in Figure 1b, however the figure also shows a stronger discrimination between M441 and T441. It is noted that the sentence prior to the sentence relied on by applicant in the Hamda reference states ". . .and one or several mismatches within antisense strands(Nos. 15-18 in Fig. 4) significantly reduces RNAi effects." The references do not teach complete discrimination, but each reference does indeed shown that siRNA mismatches provide for target discrimination. The observations provided in these references are not

different from the observation of applicant specification. Examples III-VII also show variability of siRNA discrimination. Indeed only one of applicant exemplified siRNA sequences showed 50% inhibition of a mutant with little or no effect on wt expression.

Applicant asserts that the prior art references that they rely one show that target discrimination was highly unpredictable at the time of invention. It is noted that it does not appear that target discrimination was unpredictable. The art cited, in the examiners opinion establish that one in the art would have a reasonable expectation of success in making siRNA compounds capable of target discrimination. The question at hand would appear to be would one be capable of having a reasonable expectation of making a siRNA that could discriminate 100% and inhibit its mutant target by at least 50%. It would appear that applicant own specification provides no more than the prior art in this question. Applicant observed variation in inhibitory capacity and discrimination in the three siRNA and one shRNA compounds they exemplify. The prior art has shown a range of siRNA discrimination where there is little discrimination to complete discrimination of a target.

Applicant asserts that the prior art does not teach or suggest single–nucleotide discrimination amongst SOD1 alleles at the level presently claimed. Applicant offers that the siRNA exemplified in the Tuschl and Elbashir experiment provides no evidence that the introduction of a target mismatch results in anything other than a “dead” or inactive siRNA. This line of argument is not convincing since the prior art asserts that this type of mutation/mismatch provides for discrimination and further applicant offers nothing more

than a theory that the siRNA may be “dead” or inactive without providing any evidence to refute the assertions of the prior art.

Applicant argues that the antisense art of record does not provide a teaching that the selective inhibition of SOD1 alleles was at the level of the instant invention. It is noted that the antisense art is relied upon to show that allele specific discrimination was known in the art before applicant invention and furthermore that targeting even the recited alleles of SOD1 in the instant invention were known in the art before applicants invention.

The prior art taken as a whole shows the instant invention to be obvious at the time of invention even at the levels instantly claimed. For example the prior art has taught to target alleles of SOD1 selectively utilizing antisense oligonucleotides. The art has taught that siRNA compounds can provide a higher level of discrimination than that of antisense compounds. Since the prior art has taught to target even the recited alleles with antisense compounds it is not inventive to utilize a new compound that the prior art indicates is a better compound for such methods. The prior art, evidenced by applicant citations in their response filed 4/09/2010, does show variability in the level of inhibition and discrimination when utilizing mismatched siRNAs. This is not unlike what is shown in applicant own specification. It was well known in the art that there are variations in the effectiveness of any particular siRNA, however the art has shown that one in the art is capable of screening siRNA compounds to find an siRNA that is optimized for its particular application. Since one in the art has been taught that siRNA can discriminate between alleles and since one in the art was apprised of variability, it would not be

unexpected to see differences in siRNA effectiveness for any particular target. One in the art would be capable of screening for siRNA compounds that are optimized. The fact that even the particular alleles of SOD1 recited in the claims are taught to be targets for allele discrimination it would not be inventive to screen for an optimized siRNA targeting that site.

The invention as a whole would therefore have been *prima facie* obvious to one in the art at the time the invention was made.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SEAN MCGARRY whose telephone number is (571)272-0761. The examiner can normally be reached on M-Th (6:00-4:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Heather Calamita can be reached on (571) 272-2876. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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